Patent
Attorney's Docket No. 010830-116

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
GUERIN-MARCHAND et al.	)	Group Art Unit: 1641
Application No.: Divisional of 08/462,625	)	Examiner: L. Scheiner
Filed: April 19, 2001	)	
For: PEPTIDE SEQUENCES SPECIFIC FOR THE HEPATIC STAGES OF P. FALCIPARUM BEARING EPITOPES CAPABLE OF STIMULATING THE T LYMPHOCYTES	)	

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE SPECIFICATION:

On page 1, before line 1 and after the Title, please insert the following heading:

-- **CROSS-REFERENCE TO RELATED APPLICATIONS**

This application is a divisional of Application Serial No. 08/462,625, filed on June 5, 1995. --.

Kindly replace the paragraph on page 2, line 37 to page 3, line 3 with the following:

-- These repetitive motifs (SEQ ID NO: 1) of 17 amino acids are represented by the formula:

Leu-Ala-Lys-Glu-Lys-Leu-Gln-X-Gln-Gln-Ser-Asp-Leu-Glu-Gln-Glu-Arg

in which X is Glu or Gly. --.

Kindly replace the paragraphs on page 3, line 15 to page 4, line 14 with the following:

-- **BRIEF DESCRIPTION OF THE DRAWINGS**

Reference will be made in what follows to the Figures in which:

- Figure 1 (SEQ ID NO: 31) presents a recombinant protein of the invention of 316 amino acids, designated hereafter as antigen 536 or protein LSA-R-NR,

- Figure 2 (SEQ ID NO: 32) provides the nucleotide sequence of one of the recombinant nucleic acids studied (clone DG536) and which codes for the polypeptide LSA-R-NR,

- Figure 3 (SEQ ID NO: 24) presents a polypeptide of the invention of 151 amino acids, designated hereafter as antigen 729S,

- Figure 4 (SEQ ID NO: 33) corresponds to the nucleotide sequence of the clone DG729S which codes for the polypeptide of figure 3 (EcoRI linkers in bold type),

- Figure 5 presents the polypeptide sequences (SEQ ID NOS: 23 and 26-28) of the antigens LSA-Ter, 729S-NRI, 729S-NRII, 729S-Rep,

- Figure 6 (SEQ ID NO: 34) presents the 5' end of the nucleotide sequence of the LSA gene,

- Figures 7A-7C (SEQ ID NOS: 35-37) presents the coding sequence of the 5' end of the LSA gene and the corresponding polypeptide sequence,

- Figure 8 (SEQ ID NO: 38) describes the 3' end of the LSA gene,

- Figures 9A-9D (SEQ ID NOS: 39-42) gives the sequence of the 3' end of the LSA gene as well as the corresponding polypeptide sequence,

- Figures 10A-10D (SEQ ID NOS: 43-46) repeats the sequences given in Figures 9A-9D, up to the termination codon stop and the terminal amino acid.

Thus, the present invention relates to any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the amino acid sequence shown in Figures 9A-9D or Figures 10A-10D, and corresponds to the 3' end of the LSA gene.

More particularly, the subject of the invention is any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is

represented by all or part of the sequence of the last 279 amino acids shown in Figures 10A-10D, this amino acid sequence being optionally preceded by all or part of one or more of the sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula: --.

Kindly replace the paragraph on page 5, line 6 to line 18 with the following:

-- Consequently, the invention relates more particularly to any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the following amino acid sequence (SEQ ID NO: 19):

RKADTKKNLERKKEHGDILAEDLYGRLEIPAIELPS

ENERGYYIPHQSSLPQDNRGNSRDSKEISIIEKTNR

ESITTNVEGRRDIHKGHLEEKDGSIKPEQKEDKS

this amino acid sequence being optionally preceded by all or part of one or more sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula: --.

Kindly replace the paragraphs on page 6, line 16 to line 34 with the following:

-- The invention relates more particularly to any polypeptide characterized by all or part of the following amino acid sequence (SEQ ID NO: 20):

LQEQQRDLEQRKADTKKNLERKKEHGDILAEDLYGRLEIPAIELPSENERGYY

IPHQSSLPQDNRGNSRDSKEISIIEKTNRESITTNVEGRRDIHKGHLEEKKDG

SIKPEQKEDKS

A preferred polypeptide of the invention is represented by all or part of the following amino acid sequence (SEQ ID NO: 21):

DTKKNLERKKEHGDILAEDLYGRLEIP

(this polypeptide being designated hereafter by the expression LSA-NR (LSA-non-repeated), or also by any sequence derived from the preceding sequence and modified by the substitution of maximally 40% of the amino acids while retaining its physiological activity such as the induction of a response of the T lyphocytes, in particular the cytotoxic T lymphocytes.

Another particularly preferred polypeptide of the invention is characterized by all or part of the following amino acid sequence (SEQ ID NO: 22):

ERRAKEKLQEQQRDLEQRKADTKK

(this polypeptide being designated hereafter by the expression LSA-J, or LSA-junction, since it overlaps the repetitive part and the non-repetitive part of the molecule shown in Figure 1). --.

Kindly replace the paragraphs on page 7, line 1 to line 27, with the following:

-- Another preferred peptide, designated LSA-TER, is the following (SEQ ID NO: 23):

NSRDSKEISIIEKTNRESITTNVEGRRDIHK

These last three polypeptides are more particularly useful on account of the amphipaticity which characterizes them, and because of their three-dimensional conformation according to the predictions made by the procedure of Chou and Fassmann.

The subject of the invention is also any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitope(s) characteristic of a protein produced at the sporozoite, hepatic and blood (erythrocytic) stages of P. falciparum, and more particularly bearing one or more T epitopes, characterized in that this peptide sequence is represented by all or part of the following amino acid sequence (SEQ ID NO: 24):

RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHNVEEKVE
ESVEENDEESVEENVEENVEENDDGSVASSVEESIASSVDESIDSSIEENVAP
TVEEIVAPTVEEIVAPSVVEKCAPSVEESVAPSVEESVAEMLKER

shown in Figure 3 and designated hereafter as the polypeptide 729S.

More particularly, the subject of the invention is the amino acid sequence derived from the preceding sequence and characterized by all or part of the following amino acid sequence (SEQ ID NO: 25):

RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHN

According to another advantageous embodiment of the invention, sequences of interest derived from the amino acid sequence of the polypeptide 729S are the following (SEQ ID NOS: 26-28):

- DELFNELLNSVDVNGEVKENILEESQ,

- LEESQVNDDIFSNSLVKSVQQEQQHNV,

- VEKCAPSVEESVAPSVEESVAEMLKER. --.

Kindly replace the paragraphs starting on page 8, line 1 to line 16 with the following:

-- The subject of the invention is also any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, characterized in that this peptide sequence is represented by all or part of the amino acid sequence shown in Figures 7A-7C

Consequently, the subject of the invention is more particularly any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and bearing more particularly all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the sequence of the first 153 amino acids shown in Figures 7A-7C, this amino acid sequence being optionally followed by all or part of one or more sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula: --.

Kindly replace the paragraph starting on page 9, line 6 to page 10, line 6 with the following:

-- The invention also relates to any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and bearing more particularly all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence comprises successively:

- all or part of the sequence of the first 153 amino acids shown in Figures 7A-7C,

- optionally, all or part of one or more of the sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula:

$X_1DLEQX_2RX_3AKEKLQX_4QQ$

$QX_1DLEQX_2RX_3AKEKLQX_4Q$

$QQX_1DLEQX_2RX_3AKEKLQX_4$

$X_4QQX_1DLEQX_2RX_3AKEKLQ$

$QX_4QQX_1DLEQX_2RX_3AKEKL$

$LQX_4QQX_1DLEQX_2RX_3AKEK$

$KLQX_4QQX_1DLEQX_2RX_3AKE$

$EKLQX_4QQX_1DLEQX_2RX_3AK$

$KEKLQX_4QQX_1DLEQX_2RX_3A$

$AKEKLQX_4QQX_1DLEQX_2RX_3$

$X_3AKEKLQX_4QQX_1DLEQX_2R$

$RX_3AKEKLQX_4QQX_1DLEQX_2$

$X_2RX_3AKEKLQX_4QQX_1DLEQ$

$QX_2RX_3AKEKLQX_4QQX_1DLE$

$EQX_2RX_3AKEKLQX_4QQX_1DL$

$LEQX_2RX_3AKEKLQX_4QQX_1D$

$DLEQX_2RX_3AKEKLQX_4QQX_1$

in which:

° X_1 is "Ser" or "Arg",

° X_2 is "Glu" or "Asp"

° X_3 is "Arg" or "Leu"

° X_4 is "Glu" or "Gly"

- and all or part of the last 279 amino acids shown in Figures 10A-10D. --.

Kindly replace the paragraph on page 12, line 1 to line 22 with the following:

-- The invention also relates to any sequence of nucleotides which codes for a polypeptide identical with, or one similar from the point of view of both structure and antigenic properties to, those of the invention, this sequence being capable of hybridizing with all or part of the nucleotide sequence defined by the nucleotides situated at the positions 597 to 949 of figure 2, or with all or part of the nucleotide sequence of Figure 4 or the sequences complementary to these latter, under the following conditions:

- pre-treatment (pre-hybridization) of the nitrocellulose filter supporting the nucleic acid fragment to be tested with hybridization buffer (composed of 6 SSC, 5x Denhardt's, 0.5% SDS, 100 μg/l denatured, sonicated salmon sperm DNA) this operation being carried out at 65°C for 1 hour;

- replacement of the hybridization buffer in contact with the support to which the nucleic acid fragment is now bound by hybridization buffer of the same composition and addition of the above-mentioned sequence shown in Figure 2 (SEQ ID NO: 32) or Figure 4 (SEQ ID NO: 33) as probe, in particular radioactively labelled, and denatured beforehand;

- incubation of the said nucleic acid fragment bound to the support in this incubation buffer with the above-mentioned sequence shown in Figure 2 (SEQ ID NO: 32) or Figure 4 (SEQ ID NO: 33) at 65°C for a period of about 1 hour;

- the removal of the buffer containing the probe not bound by two successive washings of 30 minutes each with a buffer solution composed of 2 x SSC and 0.5% SDS at 65°C. --.

Kindly replace the paragraph on page 14, line 24 to line 26 with the following:

-- As examples of DNA or RNA primers according to the invention, mention should be made of the following sequences (SEQ ID NOS: 29 and 30):

3'→5: TTTCGCTAGATCTTGTT & TCTAAATAGAAGAAA --.

Kindly replace the paragraph on page 19, line 10 to line 18 with the following:

-- In fact, as will be described more particularly with the aid of examples of molecules according to the invention in the detailed description which follows, the molecules according to the invention which contain all or part of the amino acid sequence comprised between the positions 200 and 316 shown in Figure 1 (SEQ ID NO: 31), react specifically with the antibodies or the lymphocytes directed against the B and/or T epitopes of the antigens produced at the hepatic stage of P. falciparum, but not with the antibodies directed against other antigens produced by P. falciparum or against antigens produced by other species of Plasmodium. --.

Kindly replace the paragraphs on page 19, line 24 to page 20, line 2 with the following:

-- These molecules according to the invention comprising all or part of the peptide sequence shown in Figure 3 (SEQ ID NO: 24) are not recognized by the former antibodies which react specifically with all or part of the polypeptide defined by the amino acids situated at the positions 200 to 316 in Figure 1.

On the other hand, the polypeptides corresponding to all or part of the peptide sequence shown in figure 3 (SEQ ID NO: 24) are recognized by antibodies which react specifically with antigens localized on the surface of sporozoites (derived from different strains of P. falciparum) as well as with antigens of the hepatic schizonts and the blood schizonts, and finally with the surface of the sporozoites of P. yoelii but not of P. berghei.

It should also be emphasized that the antibodies which recognize specifically the polypeptides corresponding to all or part of the peptide sequence shown in Figure 3 (SEQ ID NO: 24) are capable of blocking completely the entry of the sporozoites of P. yoelii into hepatic cells of rodents in vitro, unlike the antibodies directed against the circumsporozoite protein of P. yoelii and of P. falciparum. --.

Kindly replace the paragraph on page 22, at line 22 to line 24 with the following:

-- As examples of nucleotide probes of the invention, mention should be made of the following sequences:

3'→5' : TTTCGCTAGCTCTTGTT & TCTAAATAGAAGAAA --.

Kindly replace the paragraphs on page 29, line 4 to line 19 with the following:

-- The insert of 951 base pairs was purified and recloned in the bacteriophage M13 mp19. The DNA sequence and the genomic organization of the LSA gene were then determined. Figure 1 (SEQ ID NO: 31) shows that the clone contains a sequence of 209 amino acids at the 5' end corresponding to a series of 12 repeats of 17 amino acids, similar to that described in the article by Guérin-Marchand et al. (Nature, mentioned above) and then contains a set of 106 amino acids, the structure of which is not repetitive.

As can be seen in Figure 1 (SEQ ID NO: 31), the motif of 17 amino acids is in two repeats (cf. motif corresponding to the positions 35 to 51, and that corresponding to the positions 137 to 153 of figure 1) identical with that described in the article by Guérin-Marchand et al. and the other repeats exhibit a substitution of a leucine by an arginine (cf. positions 8, 59, 76, 110, 127, 161, 178 and 195 of Figure 1) (SEQ ID NO: 31), a substitution of a glutamic acid by an aspartic acid (cf. positions 23 and 91 of Figure 1 (SEQ ID NO: 31)) as well as a substitution of a serine by an arginine (cf. position 205 of Figure 1 (SEQ ID NO: 31)). --.

Please insert the attached paper copy of the "Sequence Listing" between the last page of the Disclosure (page 35) and the first page of the Claims. Please renumber the pages accordingly.

IN THE CLAIMS:

Please cancel claims 1, 19 and 21 without prejudice or disclaimer of the subject matter recited therein.

Please add claims 27 through 38 as follows:

27. (New) An *in vitro* diagnostic method for malaria in an individual comprising placing a tissue or a biological fluid taken from an individual in contact with a molecule or polypeptide composition, wherein said molecule or polypeptide composition comprises one or more peptide sequences bearing all or part of one or more T epitopes of the proteins resulting from the infectious activity of *P. falciparum*, under conditions allowing an *in vitro* immunological reaction to occur between said composition and the antibodies that may be present in the tissue or biological fluid, and *in vitro* detection of the antigen-antibody complexes formed.

28. (New) The molecule or polypeptide composition according to claim 27 wherein said molecule or polypeptide composition further comprises B epitopes of the proteins resulting from the infectious activity of *P. falciparum*.

29. (New) A kit for the *in vitro* diagnosis of malaria according to claim 27, wherein said kit comprises:

a) one or more molecule or polypeptide compositions, wherein said molecule or polypeptide compositions comprise one or more peptide sequences bearing all or part of one or more T epitopes of the proteins resulting from the infectious activity of *P. falciparum*;

b) the reagents for making up a suitable medium for carrying out the immunological reaction; and

c) the reagents allowing the detection of the antigen-antibody complexes produced as a result of the immunological reaction.

30. (New) The kit according to claim 29, wherein said reagents in step c) bear a label or are recognized by a labeled reagent.

31. (New) A polypeptide comprising at least one T epitope from a liver-stage specific protein produced by *P. falciparum*.

32. (New) The polypeptide according to claim 31, wherein said T epitope has an amino acid sequence selected from the group of the amino acid sequence of Figures 9A-9D and the amino acid sequence of Figures 10A-10D.

33. (New) The polypeptide according to claim 31, wherein said T epitope consists of the amino acid sequence of SEQ ID NO. 19.

34. (New) The polypeptide according to claim 33, wherein said T epitope is preceded by one or more of the amino acid sequences of SEQ ID NOS. 2 to 18, wherein X_1 is Ser or Arg; X_2 is Glu or Asp; X_3 is Arg or Leu and X_4 is Glu or Gly.

35. (New) The polypeptide of claim 31, further comprising at least one B epitope from a liver-stage specific protein produced by *P. falciparum*.

36. (New) A vaccine composition directed against malaria comprising a molecule having one or more peptide sequences bearing all or part of one or more T epitopes resulting from the infectious activity of *P. falciparum* in the hepatic cells.

37. (New) The vaccine composition directed against malaria according to claim 36, wherein said T epitope is selected from the group of: an amino acid sequence of Figures 9A-9D, an amino acid sequence of Figures 10A-10D and an amino acid sequence of SEQ ID NO. 19.

38. (New) The vaccine composition directed against malaria according to claim 37, wherein said T epitope is preceded by one or more of the amino acid sequences of SEQ ID NOS. 2 to 18, wherein X_1 is Ser or Arg; X_2 is Glu or Asp; X_3 is Arg or Leu and X_4 is Glu or Gly.

REMARKS

Entry of the foregoing and favorable consideration of the subject application, in light of the following remarks, are respectfully requested.

By the present preliminary amendment, the specification has been amended to insert the attached paper copy of the Sequence Listing between the last page of the Disclosure (page 35) and the first page of the Claims. Further, the specification has been amended to insert the appropriate headings throughout the specification, and to insert the appropriate sequence listing identifiers throughout the specification. Additionally, in view of the formal drawings filed concurrently herewith, the specification has also been further amended to correct certain figure designations throughout the specification as well as in the Brief Description of the Drawings section of the specification.

Claims 1, 19 and 21 have been canceled without prejudice or disclaimer of the subject matter recited therein, and new claims 27-38 have been added. Support for new claims 27-28 and 29-30 can be found in prior claims 19 and 21, respectively. Support for new claims 31-32 and 33-34 can be found in originally filed claims 2 and 3, respectively. Support for new claim 35 can be found in originally filed claim 1, and support for new claims 36-38 can be found in originally filed claim 3 and on pages 22-23 of the specification. Accordingly, no new matter has been added.

In the event that there are any questions relating to this Preliminary Amendment, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that issuance of this application may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
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Page 1, Before Line 1 and After the Title

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. Application Serial No. 08/462,625 filed June 5, 1995.

Page 2, Paragraph Beginning at Line 37 to Page 3

These repetitive motifs (SEQ ID NO: 1) of 17 amino acids are represented by the formula:

Leu-Ala-Lys-Glu-Lys-Leu-Gln-X-Gln-Gln-Ser-Asp-Leu-Glu-Gln-Glu-Arg

in which X is Glu or Gly.

Page 3, Paragraph Beginning at Line 15 to Page 4

BRIEF DESCRIPTION OF THE DRAWINGS

Reference will be made in what follows to the Figures in which:

- Figure 1 (SEQ ID NO: 31) presents a recombinant protein of the invention of 316 amino acids, designated hereafter as antigen 536 or protein LSA-R-NR,
- Figure 2 (SEQ ID NO: 32) provides the nucleotide sequence of one of the recombinant nucleic acids studied (clone DG536) and which codes for the polypeptide LSA-R-NR,
- Figure 3 (SEQ ID NO: 24) presents a polypeptide of the invention of 151 amino acids, designated hereafter as antigen 729S,

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- Figure 4 (SEQ ID NO: 33) corresponds to the nucleotide sequence of the clone DG729S which codes for the polypeptide of figure 3 (EcoRI linkers in bold type),

- Figure 5 presents the polypeptide sequences (SEQ ID NOS: 23 and 26-28) of the antigens LSA-Ter, 729S-NRI, 729S-NRII, 729S-Rep,

- Figure 6 (SEQ ID NO: 34) presents the 5' end of the nucleotide sequence of the LSA gene,

- [Figure 7] Figures 7A-7C (SEQ ID NOS: 35-37) presents the coding sequence of the 5' end of the LSA gene and the corresponding polypeptide sequence,

- Figure 8 (SEQ ID NO: 38) describes the 3' end of the LSA gene,

- [Figure 9] Figures 9A-9D (SEQ ID NOS: 39-42) gives the sequence of the 3' end of the LSA gene as well as the corresponding polypeptide sequence,

- [Figure 10] Figures 10A-10D (SEQ ID NOS: 43-46) repeats the sequences given in [Figure 9] Figures 9A-9D, up to the termination codon stop and the terminal amino acid.

Thus, the present invention relates to any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the amino acid sequence shown in [Figure 9] Figures 9A-9D or Figures 10A-10D, and corresponds to the 3' end of the LSA gene.

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More particularly, the subject of the invention is any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the sequence of the last 279 amino acids shown in Figures 10A-10D, this amino acid sequence being optionally preceded by all or part of one or more of the sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula:

Page 5, Paragraph Beginning at Line 6

Consequently, the invention relates more particularly to any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the following amino acid sequence (SEQ ID NO: 19):

RKADTKKNLERKKEHGDILAEDLYGRLEIPAIELPS

ENERGYYIPHQSSLPQDNRGNSRDSKEISIIEKTNR

ESITTNVEGRRDIHKGHLEEKDGSIKPEQKEDKS

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this amino acid sequence being optionally preceded by all or part of one or more sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula:

Page 6, Paragraph Beginning at Line 16

The invention relates more particularly to any polypeptide characterized by all or part of the following amino acid sequence (SEQ ID NO: 20):

LQEQQRDLEQRKADTKKNLERKKEHGDILAEDLYGRLEIPAIELPSENERGYY

IPHQSSLPQDNRGNSRDSKEISIIEKTNRESITTNVEGRRDIHKGHLEEKKDG

SIKPEQKEDKS

A preferred polypeptide of the invention is represented by all or part of the following amino acid sequence (SEQ ID NO: 21):

DTKKNLERKKEHGDILAEDLYGRLEIP

(this polypeptide being designated hereafter by the expression LSA-NR (LSA-non-repeated), or also by any sequence derived from the preceding sequence and modified by the substitution of maximally 40% of the amino acids while retaining its physiological activity such as the induction of a response of the T lyphocytes, in particular the cytotoxic T lymphocytes.

Another particularly preferred polypeptide of the invention is characterized by all or part of the following amino acid sequence (SEQ ID NO: 22):

ERRAKEKLQEQQRDLEQRKADTKK

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(this polypeptide being designated hereafter by the expression LSA-J, or LSA-junction, since it overlaps the repetitive part and the non-repetitive part of the molecule shown in Figure 1).

Page 7, Paragraph Beginning at Line 1

Another preferred peptide, designated LSA-TER, is the following (SEQ ID NO: 23):

NSRDSKEISIIEKTNRESITTNVEGRRDIHK

These last three polypeptides are more particularly useful on account of the amphipaticity which characterizes them, and because of their three-dimensional conformation according to the predictions made by the procedure of Chou and Fassmann.

The subject of the invention is also any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitope(s) characteristic of a protein produced at the sporozoite, hepatic and blood (erythrocytic) stages of P. falciparum, and more particularly bearing one or more T epitopes, characterized in that this peptide sequence is represented by all or part of the following amino acid sequence (SEQ ID NO: 24):

RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHNVEEKVE

ESVEENDEESVEENVEENVEENDDGSVASSVEESIASSVDESIDSSIEENVAP

TVEEIVAPTVEEIVAPSVVEKCAPSVEESVAPSVEESVAEMLKER

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shown in Figure 3 and designated hereafter as the polypeptide 729S.

More particularly, the subject of the invention is the amino acid sequence derived from the preceding sequence and characterized by all or part of the following amino acid sequence (SEQ ID NO: 25):

RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHN

According to another advantageous embodiment of the invention, sequences of interest derived from the amino acid sequence of the polypeptide 729S are the following (SEQ ID NOS: 26-28):

- DELFNELLNSVDVNGEVKENILEESQ,
- LEESQVNDDIFSNSLVKSVQQEQQHNV,
- VEKCAPSVEESVAPSVEESVAEMLKER.

Page 8, Paragraph Beginning at Line 1

The subject of the invention is also any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, characterized in that this peptide sequence is represented by all or part of the amino acid sequence shown in [Figure 7] Figures 7A-7C.

Consequently, the subject of the invention is more particularly any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one

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or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and bearing more particularly all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the sequence of the first 153 amino acids shown in [Figure 7] Figures 7A-7C, this amino acid sequence being optionally followed by all or part of one or more sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula:

Page 9, Paragraph Beginning at Line 6 to Page 10

The invention also relates to any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and bearing more particularly all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence comprises successively:

- all or part of the sequence of the first 153 amino acids shown in [Figure 7] Figures 7A-7C,

- optionally, all or part of one or more of the sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula:

$X_1DLEQX_2RX_3AKEKLQX_4QQ$

$QX_1DLEQX_2RX_3AKEKLQX_4Q$

$QQX_1DLEQX_2RX_3AKEKLQX_4$

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$X_4QQX_1DLEQX_2RX_3AKEKLQ$

$QX_4QQX_1DLEQX_2RX_3AKEKL$

$LQX_4QQX_1DLEQX_2RX_3AKEK$

$KLQX_4QQX_1DLEQX_2RX_3AKE$

$EKLQX_4QQX_1DLEQX_2RX_3AK$

$KEKLQX_4QQX_1DLEQX_2RX_3A$

$AKEKLQX_4QQX_1DLEQX_2RX_3$

$X_3AKEKLQX_4QQX_1DLEQX_2R$

$RX_3AKEKLQX_4QQX_1DLEQX_2$

$X_2RX_3AKEKLQX_4QQX_1DLEQ$

$QX_2RX_3AKEKLQX_4QQX_1DLE$

$EQX_2RX_3AKEKLQX_4QQX_1DL$

$LEQX_2RX_3AKEKLQX_4QQX_1D$

$DLEQX_2RX_3AKEKLQX_4QQX_1$

in which:

° X_1 is "Ser" or "Arg",

° X_2 is "Glu" or "Asp"

° X_3 is "Arg" or "Leu"

° X_4 is "Glu" or "Gly"

- and all or part of the last 279 amino acids shown in [Figure 10] Figures 10A-10D.

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Page 12, Paragraph Beginning at Line 1

The invention also relates to any sequence of nucleotides which codes for a polypeptide identical with, or one similar from the point of view of both structure and antigenic properties to, those of the invention, this sequence being capable of hybridizing with all or part of the nucleotide sequence defined by the nucleotides situated at the positions 597 to 949 of figure 2, or with all or part of the nucleotide sequence of Figure 4 or the sequences complementary to these latter, under the following conditions:

- pre-treatment (pre-hybridization) of the nitrocellulose filter supporting the nucleic acid fragment to be tested with hybridization buffer (composed of 6 SSC, 5x Denhardt's, 0.5% SDS, 100 μg/l denatured, sonicated salmon sperm DNA) this operation being carried out at 65°C for 1 hour;
- replacement of the hybridization buffer in contact with the support to which the nucleic acid fragment is now bound by hybridization buffer of the same composition and addition of the above-mentioned sequence shown in Figure 2 (SEQ ID NO: 32) or Figure 4 (SEQ ID NO: 33) as probe, in particular radioactively labelled, and denatured beforehand;
- incubation of the said nucleic acid fragment bound to the support in this incubation buffer with the above-mentioned sequence shown in Figure 2 (SEQ ID NO: 32) or Figure 4 (SEQ ID NO: 33) at 65°C for a period of about 1 hour;
- the removal of the buffer containing the probe not bound by two successive washings of 30 minutes each with a buffer solution composed of 2 x SSC and 0.5% SDS at 65°C.

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Page 14, Paragraph Beginning at Line 24

As examples of DNA or RNA primers according to the invention, mention should be made of the following sequences (SEQ ID NOS: 29 and 30):

3'→5: TTTCGCTAGATCTTGTT & TCTAAATAGAAGAAA.

Page 19, Paragraph Beginning at Line 10

In fact, as will be described more particularly with the aid of examples of molecules according to the invention in the detailed description which follows, the molecules according to the invention which contain all or part of the amino acid sequence comprised between the positions 200 and 316 shown in Figure 1 (SEQ ID NO: 31), react specifically with the antibodies or the lymphocytes directed against the B and/or T epitopes of the antigens produced at the hepatic stage of P. falciparum, but not with the antibodies directed against other antigens produced by P. falciparum or against antigens produced by other species of Plasmodium.

Page 19, Paragraph Beginning at Line 24 to Page 20

These molecules according to the invention comprising all or part of the peptide sequence shown in Figure 3 (SEQ ID NO: 24) are not recognized by the former antibodies which react specifically with all or part of the polypeptide defined by the amino acids situated at the positions 200 to 316 in Figure 1.

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On the other hand, the polypeptides corresponding to all or part of the peptide sequence shown in figure 3 (SEQ ID NO: 24) are recognized by antibodies which react specifically with antigens localized on the surface of sporozoites (derived from different strains of P. falciparum) as well as with antigens of the hepatic schizonts and the blood schizonts, and finally with the surface of the sporozoites of P. yoelii but not of P. berghei.

It should also be emphasized that the antibodies which recognize specifically the polypeptides corresponding to all or part of the peptide sequence shown in Figure 3 (SEQ ID NO: 24) are capable of blocking completely the entry of the sporozoites of P. yoelii into hepatic cells of rodents in vitro, unlike the antibodies directed against the circumsporozoite protein of P. yoelii and of P. falciparum.

Page 22, Paragraph Beginning at Line 22

As examples of nucleotide probes of the invention, mention should be made of the following sequences:

3'→5' : TTTCGCTAGCTCTTGTT & TCTAAATAGAAGAAA--.

Page 29, Paragraph Beginning at Line 4

The insert of 951 base pairs was purified and recloned in the bacteriophage M13 mp19. The DNA sequence and the genomic organization of the LSA gene were then determined. Figure 1 (SEQ ID NO: 31) shows that the clone contains a sequence of 209

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amino acids at the 5' end corresponding to a series of 12 repeats of 17 amino acids, similar to that described in the article by Guérin-Marchand et al. (Nature, mentioned above) and then contains a set of 106 amino acids, the structure of which is not repetitive.

As can be seen in Figure 1 (SEQ ID NO: 31), the motif of 17 amino acids is in two repeats (cf. motif corresponding to the positions 35 to 51, and that corresponding to the positions 137 to 153 of figure 1) identical with that described in the article by Guérin-Marchand et al. and the other repeats exhibit a substitution of a leucine by an arginine (cf. positions 8, 59, 76, 110, 127, 161, 178 and 195 of Figure 1) (SEQ ID NO: 31), a substitution of a glutamic acid by an aspartic acid (cf. positions 23 and 91 of Figure 1 (SEQ ID NO: 31)) as well as a substitution of a serine by an arginine (cf. position 205 of Figure 1 (SEQ ID NO: 31)).

Between the last page of the Disclosure (page 35) and the first page of the Claims, insert the attached paper copy of the "Sequence Listing".